PATENT
PDNO 10031463-1

APPARATUS AND METHOD FOR SIMULTANEOUSLY CONDUCTING MULTIPLE CHEMICAL REACTIONS

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RELATED APPLICATIONS

This application is a ***continuation-in-part*** of U.S. Patent Application Ser. No. 09/938,909, filed August 24, 2001, the disclosure of which is incorporated herein by reference in its entirety.

BACKGROUND

1. Technical Field

This invention relates to simultaneously reacting multiple chemical samples using arrays. In particular, the invention relates to an apparatus for and a method of conducting multiple chemical reactions or assays simultaneously using microarray technology for diagnostic, therapeutic and analytical applications.

2. Description of Related Art

Devices for processing multiple biological assays are known and typically comprise reaction vessels having a plurality of reaction wells, each well has sidewalls and a closed end or bottom and an array of chemical samples fixed to the closed end. The end opposite to the closed end is open to access the reaction well for performing the assays with the array. Conventional microtiter plates are examples of conventional reaction vessels.

The conventional reaction vessels are not self-contained (e.g., gas tight or fluid tight). Therefore, special handling and processing equipment must be used to perform assays using these conventional reaction vessels in order to provide stable handling and to control assay conditions, such as atmosphere, temperature, contamination, and prevent loss of sample or reagents, and the like. The special handling and processing equipment is expensive and not conducive to increased throughput, without added expense. Moreover, additional materials and assembly steps are needed if one skilled in the art wanted to seal the conventional reaction vessels to render them gas or fluid tight.

Thus, it would be advantageous to simultaneously conduct multiple chemical reactions in a reaction vessel that is gas, liquid and/or fluid tight without the need for cover plates and/or caps and the associated additional assembly steps. Such an apparatus and a method could decrease cost and increase throughput in the art of performing multiple assays.

BRIEF SUMMARY

In an embodiment of the invention, an apparatus to simultaneously conduct multiple chemical reactions is provided. The apparatus comprises a plate having a plurality of spatially arranged wells. A well has a deformable closed end, a sidewall, and an open end opposite to the closed end. The apparatus further comprises an array having a plurality of sets of chemical samples bound to a surface of the array in a spatial arrangement similar to the spatially arranged of the wells. When assembled together, the array covers the open ends of the wells in the plate to form a reaction assembly having a plurality of closed cells that is one or more of gas tight, liquid tight and fluid tight.

In another embodiment of the invention, an apparatus to simultaneously conduct multiple chemical reactions is provided. The apparatus comprises a plate having a plurality of spatially arranged wells. A well has a closed end, a sidewall, and an open end opposite to the closed end. The apparatus further comprises a multi array device. The multi array device comprises a foundation support, a plurality of prongs extending from the foundation support in a spatial arrangement similar to the spatial arrangement of the wells, and sets of chemical reactants bound to distal ends of the prongs. When assembled together, the multi array device covers the open ends of the wells to form a reaction assembly having a plurality of closed cells that is one or more of gas tight, liquid tight, and fluid tight.

In another embodiment of the invention, a method of simultaneously conducting multiple chemical reactions is provided. The method comprises providing an array of sets of chemical reactants bound to an array surface; and providing a plate of wells spatially arranged similar to the array of sets. A well has a deformable closed end and an open end opposite the closed end. When assembled together, the array covers the open ends of the wells to form a reaction assembly having a plurality of closed cells

that is one or more of a gas tight, a liquid tight, and a fluid tight to simultaneously conduct multiple chemical reactions.

In still another embodiment of the invention, a kit to simultaneously conduct multiple different assays of biological materials is provided. The kit comprises an array having a plurality of sets of a first biological material bound to an array substrate in a spatial arrangement. The kit further comprises a plate having a plurality of spatially arranged wells in the plate. The wells are closed at one end with a flexible end wall and open at an opposite end. When assembled together, the array covers the open ends of the wells in the plate to form a reaction assembly having multiple closed reaction cells that are one or more of gas, fluid or liquid tight. Depending on the embodiment, the plate may be a flexible multi-well plate and the array may be a multi array device.

Embodiments of the present invention provide a reaction assembly to perform simultaneous multiple chemical reactions that use an array substrate to cap or close the open ends of the wells in a plate. The reaction assembly is sealed to provide one or more of a gas, a liquid, or fluid tight seal. The seal may use material characteristics of one or more of the plate, the array and a gasket to achieve the seal. Moreover or alternatively, the seal may use one or more of pressure and an adhesive to achieve the seal. Conventional covers or caps and the assembly thereof are essentially avoided with the embodiments of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The various features of the embodiments of the present invention may be more readily understood with reference to the following detailed description taken in conjunction with the accompanying drawings, where like reference numerals designate like structural elements, and in which:

Figure 1 illustrates a side view of an embodiment of a reaction assembly apparatus that includes a flexible multi-well plate according to an embodiment of the present invention.

Figure 2 illustrates a cross sectional side view of an embodiment of a flexible multi-well plate according to an embodiment of the present invention.

Figure 3 illustrates a cross sectional side view of another embodiment of a flexible multi-well plate according to an embodiment of the present invention.

Figure 4 illustrates a side view of an embodiment of the reaction assembly apparatus that includes the flexible multi-well plate of Figure 2.

Figure 5 illustrates a side view of another embodiment of the reaction assembly apparatus that includes the flexible multi-well plate of Figure 3.

Figure 6 illustrates a side view of an embodiment of a reaction assembly that includes a multi array device according to an embodiment of the present invention.

Figure 7 illustrates a perspective view of an embodiment of a multi array device according to an embodiment of the present invention.

Figure 8 illustrates a flow chart of an embodiment of a method of simultaneously conducting chemical reactions according to an embodiment of the present invention.

Figure 9 illustrates a plan view of a portion of a major surface of an embodiment of a gasket for sealing a reaction assembly according to an embodiment of the present invention.

Figure 10 illustrates a side view of an embodiment of a reaction assembly apparatus that includes the gasket of Figure 9 according to an embodiment of the present invention.

DETAILED DESCRIPTION

Definitions

The following terms are intended to have the following general meanings as they are used herein, unless otherwise indicated below. Any definitions not provided herein may be provided in a reference cited and incorporated by reference herein. Moreover, any definition provided herein may be supplemented by a reference cited and incorporated by reference herein.

Gas – a substance or a mixture of substances, exhibiting zero surface tension and typically having low viscosity, including for example air, nitrogen, argon, helium, or superheated steam, and also including aerosols such as smoke and clouds of water vapor at a temperature less than the local boiling temperature for water. Gases are

also considered herein to include gaseous plasmas containing charged particles.

Liquid – a substance or mixture of substances exhibiting surface tension and typically having higher viscosity than a gas, including for example water, oil, and liquid mercury. Liquids are also considered herein to include wet slurries containing finely divided powders in liquids.

Fluid – a substance exhibiting viscous flow characteristics, including both liquids and gases, and also including dry powders such as graphite, flour, and sand. A special case of a fluid is liquid helium exhibiting superfluid flow having zero viscosity.

Gas tight, liquid tight, and fluid tight seals – To be useful, a seal should be one or more of gas tight, liquid tight, and fluid tight against the flow of the particular substances to be contained by the seal. If gas tight against the flow of a particular gas, a seal will typically be tight also against the flow of liquids and powders. If liquid tight against the flow of a particular liquid, a seal may permit the flow of most gases. If fluid tight against the flow of a particular powder, a seal may permit the flow of most liquids and gases. However, a fluid tight seal can, at the extreme, be tight against the flow of all fluids including gases and liquids.

Nucleic acid – a high molecular weight material that is a polynucleotide or an oligonucleotide of DNA or RNA.

Polynucleotide – a compound or composition that is a polymeric nucleotide or nucleic acid polymer. The polynucleotide may be a natural compound or a synthetic compound. In the context of an assay, the polynucleotide can have from about 20 to 5,000,000 or more nucleotides. The larger polynucleotides are generally found in the natural state. In an isolated state the polynucleotide can have about 30 to 50,000 or more nucleotides, usually about 100 to 20,000 nucleotides, more frequently 500 to 10,000 nucleotides. It is thus obvious that isolation of a polynucleotide from the natural state often results in fragmentation. The polynucleotides include nucleic acids, and fragments thereof, from any source in purified or unpurified form including DNA, double-stranded or single-stranded (dsDNA and ssDNA), and RNA, including t-RNA, m-RNA, r-RNA, mitochondrial DNA and RNA, chloroplast DNA and RNA, complementary DNA (cDNA) (a single stranded DNA that is complementary to an RNA and synthesized from the RNA in vitro using reverse transcriptase), DNA/RNA

hybrids, or mixtures thereof, genes, chromosomes, plasmids, the genomes of biological materials such as microorganisms, e.g. bacteria, yeasts, viruses, viroids, molds, fungi, plants, animals, humans, and the like. The polynucleotide can be only a minor fraction of a complex mixture such as a biological sample. Also included are genes, such as hemoglobin gene for sickle-cell anemia, cystic fibrosis gene, oncogenes, and the like.

Polynucleotides include analogs of naturally occurring polynucleotides in which one or more nucleotides are modified over naturally occurring nucleotides. Polynucleotides then, include compounds produced synthetically (for example, PNA as described in U. S. Patent No. 5,948,902 and the references cited therein, all of which are incorporated herein by reference), which can hybridize in a sequence specific manner analogous to that of naturally occurring complementary polynucleotides.

The polynucleotide can be obtained from various biological materials by procedures well known in the art. The polynucleotide, where appropriate, may be cleaved to obtain a fragment that contains a target nucleotide sequence, for example, by shearing or by treatment with a restriction endonuclease or other site-specific chemical cleavage method.

For purposes of this invention, the polynucleotide, or a cleaved fragment obtained from the polynucleotide, will usually be at least partially denatured or single-stranded or treated to render it denatured or single-stranded. Such treatments are well known in the art and include, for instance, heat or alkali treatment, or enzymatic digestion of one strand. For example, double stranded DNA (dsDNA) can be heated at 90-100°C for a period of about 1 to 10 minutes to produce denatured material, while RNA produced via transcription from a dsDNA template is already single-stranded.

Oligonucleotide – a polynucleotide, usually single-stranded, usually a synthetic polynucleotide but may be a naturally occurring polynucleotide. The oligonucleotide(s) are usually comprised of a sequence of at least 5 nucleotides, usually, 10 to 100 nucleotides, preferably 20 to 60 nucleotides, and more preferably 25 to 60 nucleotides in length.

Various techniques can be employed for preparing an oligonucleotide. Such oligonucleotides can be obtained by biological synthesis or by chemical synthesis. For short sequences (up to about 100 nucleotides), chemical synthesis will frequently be more economical as compared to the biological synthesis. In addition to economy, chemical synthesis provides a convenient way of incorporating low molecular weight compounds and/or modified bases during specific synthesis steps. Furthermore, chemical synthesis is very flexible in the choice of length and region of target polynucleotides binding sequence. The oligonucleotide can be synthesized by standard methods such as those used in commercial automated nucleic acid synthesizers. Chemical synthesis of DNA on a suitably modified glass or resin can result in DNA covalently attached to the surface. This may offer advantages in washing and sample handling. For longer sequences standard replication methods employed in molecular biology can be used such as the use of M13 for single-stranded DNA as described in J. Messing (1983) *Methods Enzymol.* 101:20-78.

Other methods of oligonucleotide synthesis include phosphotriester and phosphodiester methods (Narang, et al., (1979) *Meth. Enzymol.* 68:90) and synthesis on a support (Beaucage, et al. (1981) *Tetrahedron Letters* 22:1859-1862) as well as phosphoramidite techniques (Caruthers, M. H., et al., "Methods in Enzymology," Vol. 154, pp. 287-314 (1988) and others described in "Synthesis and Applications of DNA and RNA," S. A. Narang, editor, Academic Press, New York, 1987, and the references contained therein. The chemical synthesis via a photolithographic method of spatially addressable arrays of oligonucleotides bound to glass surfaces is described by A. C. Pease, et al., *Proc. Nat. Aca. Sci. USA* (1994) 91:5022-5026. Unless otherwise noted herein, the terms nucleic acid, oligonucleotide and polynucleotide are intended to be used interchangeably.

Nucleotide - the monomeric unit of nucleic acid polymers, i.e., DNA and RNA, whether obtained from a natural source or produced synthetically, which comprises a nitrogenous heterocyclic base, which is a derivative of either a purine or pyrimidine, a pentose sugar, and a phosphate (or phosphoric acid). When the phosphate is removed, the monomeric unit that remains is a "nucleoside". Thus a nucleotide is a 5'-phosphate of the corresponding nucleoside. When the nitrogenous base is removed from the nucleotide, the monomeric unit that remains is a "phosphodiester". For the

purposes of the invention, "nucleotide" includes its corresponding nucleoside and phosphodiester, and "oligonucleotide" includes its corresponding oligonucleoside and oligophosphodiester, unless indicated otherwise. The term "nucleotide" includes "modified nucleotide" that contains a modified base, sugar or phosphate group. The modified nucleotide can be produced by a chemical modification of a nucleotide either as part of the nucleic acid polymer or prior to the incorporation of the modified nucleotide into the nucleic acid polymer. For example, the methods mentioned above for the synthesis of an oligonucleotide may be employed. In another approach, a modified nucleotide can be produced by incorporating a modified nucleoside triphosphate into the polymer chain during an amplification reaction. Examples of modified nucleotides, by way of illustration and not limitation, include dideoxynucleotides, derivatives or analogs that are biotinylated, amine modified, alkylated, fluorophore-labeled, and the like and also include phosphorothioate, phosphite, ring atom modified derivatives, and so forth.

Target – a compound to be identified, such as a test sample. In nucleic acid assays, a target is usually existing within a portion or all of a polynucleotide, usually a polynucleotide analyte. The identity of the target nucleotide sequence generally is known to an extent sufficient to allow preparation of various probe sequences hybridizable with the target material.

The target material usually contains from about 20 to 5,000 or more nucleotides, preferably 30 to 1,000 nucleotides. The target material is generally a fraction of a larger molecule or it may be substantially the entire molecule such as a polynucleotide as described above. The minimum number of nucleotides in the target material is selected to assure that the presence of a target polynucleotide in a sample is a specific indicator of the presence of polynucleotide in a sample. The maximum number of nucleotides in the target material is normally governed by several factors: the length of the polynucleotide from which it is derived, the tendency of such polynucleotide to be broken by shearing or other processes during isolation, the efficiency of any procedures required to prepare the sample for analysis (e.g. transcription of a DNA template into RNA) and the efficiency of detection and/or amplification of the target nucleotide sequence, where appropriate.

Probe – a compound used to evaluate a target, such as a chemical reactant. In nucleic acid assays, the probe is an oligonucleotide or polynucleotide employed to bind to a portion of a polynucleotide such as another oligonucleotide or a target material. The design and preparation of the nucleic acid probes are generally dependent upon the sensitivity and specificity required, the sequence of the target material and, in certain cases, the biological significance of certain portions of the target material.

Hybridization (hybridizing) and binding – in the context of nucleotide sequences these terms are used interchangeably herein. The ability of two nucleotide sequences to hybridize with each other is based on the degree of complementarity of the two nucleotide sequences, which in turn is based on the fraction of matched complementary nucleotide pairs. The more nucleotides in a given sequence that are complementary to another sequence, the more stringent the conditions can be for hybridization and the more specific will be the binding of the two sequences. Increased stringency is achieved by elevating the temperature, increasing the ratio of co-solvents, lowering the salt concentration, and the like. For the purposes of the invention, hybridization of complementary Watson/Crick base pairs of probes on the microarray and of the target material is preferred, but non Watson/Crick base pairing during hybridization may also occur.

Conventional hybridization solutions and processes for hybridization are described in J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, Ed. 2nd, 1989, vol. 1-3, incorporated herein by reference. Conditions for hybridization typically include (1) high ionic strength solution, (2) at a controlled temperature, and (3) in the presence of carrier DNA and surfactants and chelators of divalent cations, all of which are well known in the art.

Complementary – Two sequences are complementary when the sequence of one can bind to the sequence of the other in an anti-parallel sense wherein the 3'-end of each sequence binds to the 5'-end of the other sequence and each A, T(U), G, and C of one sequence is then aligned with a T(U), A, C, and G, respectively, of the other sequence, to form Watson/Crick base pairs. RNA sequences can also include complementary G=U or U=G base pairs. Non-standard or non Watson/Crick base

pairing is also possible with nucleotide complements, for instance, the sequences may be parallel to each other and complementary A=C or G=U base pairs in RNA sequences or complementary G=T or A=C base pairs in DNA sequences may occur, although are not preferred.

Substrate or surface - a porous or non-porous support material, preferably a reaction solvent-insoluble support material. The surface can have any one of a number of shapes, such as strip, plate, disk, rod, particle, including bead, and the like. The substrate can be hydrophobic or hydrophilic or capable of being rendered hydrophobic or hydrophilic and includes inorganic powders such as silica, magnesium sulfate, and alumina; natural polymeric materials, particularly cellulosic materials and materials derived from cellulose, such as fiber-containing papers, e.g., filter paper, chromatographic paper, etc.; synthetic or modified naturally occurring polymers, such as nitrocellulose, cellulose acetate, poly (vinyl chloride), polyacrylamide, cross linked dextran, agarose, polyacrylate, polyethylene, polypropylene, poly (4-methylbutene), polystyrene, polymethacrylate, poly(ethylene terephthalate), nylon, poly(vinyl butyrate), etc.; either used by themselves or in conjunction with other materials; glass available as Bioglass, ceramics, metals, anodized metal surfaces, semiconductors, coated semiconductor surfaces, and the like. Natural or synthetic assemblies such as liposomes, phospholipid vesicles, and cells can also be employed.

Common substrates used for microarrays are surface-derivatized glass or silica, or polymer membrane surfaces, as described in Z. Guo et al. (cited above) and U. Maskos, E. M. Southern, *Nucleic Acids Res.* 20, 1679-84 (1992) and E. M. Southern et al., *Nucleic Acids Res.* 22, 1368-73 (1994), both incorporated herein by reference. In modifying siliceous or metal oxide surfaces, one technique that has been used is derivatization with bifunctional silanes, i.e., silanes having a first functional group enabling covalent binding to the surface (often an Si-halogen or Si-alkoxy group, as in $SiCl_3$ or $-Si(OCH_3)_3$, respectively) and a second functional group that can impart the desired chemical and/or physical modifications to the surface to covalently or non-covalently attach ligands and/or the polymers or monomers for the biological probe array. See, for example, U. S. Patent No. 5,266,222 to Willis and U. S. Patent No. 5,137,765 to Farnsworth, each of which is incorporated herein by reference.

Immobilization of oligonucleotides on a substrate or surface may be accomplished by well-known techniques, commonly available in the literature. See, for example, A. C. Pease, et al., *Proc. Nat. Acad. Sci. USA*, 91:5022-5026 (1994); Z. Guo, R. A. Guilfoyle, A. J. Thiel, R. Wang, L. M. Smith, *Nucleic Acids Res.* 22, 5456-65 (1994); and M. Schena, D. Shalon, R. W. Davis, P. O. Brown, *Science*, 270, 467-70 (1995), each incorporated herein by reference. Substrates may be purchased with a coating, or coated, with an adsorbed polymer to facilitate linking to the surface. See for example, web site http://cmgm.stanford.edu/pbrown/protocols/1_slides.html for conventional coating protocols.

Feature - a feature is either a probe/chemical reactant or a target/chemical test sample bonded to an array substrate. The location of a feature is addressable, typically by a row and column location, or other spatial address. A feature may include a plurality or a set of 'subfeatures', where each set is a 'subarray'. For the purposes of the invention, there is a plurality of features on an array or microarray, where each feature comprises a plurality of subfeatures. However, the term 'microarray' may be characterized by some skilled in the art as comprising one or more of the subarrays of a larger array. For the purposes of the invention, the terms 'array' and 'microarray' are used interchangeably herein unless specified otherwise.

Label - a member of a signal producing system. Usually the label is part of a target or a probe, either being conjugated thereto or otherwise bound thereto or associated therewith. The label is capable of being detected directly or indirectly. Labels include (i) reporter molecules that can be detected directly by virtue of generating a signal, e.g., a fluorophore, (ii) specific binding pair members that may be detected indirectly by subsequent binding to a cognate that contains a reporter molecule, e.g., biotin-streptavidin, (iii) oligonucleotide primers that can provide a template for amplification or ligation or (iv) a specific polynucleotide sequence or recognition sequence that can act as a ligand such as for a repressor protein, wherein in the latter two instances the oligonucleotide primer or repressor protein will have, or be capable of having, a reporter molecule. Labels further include but are not limited to radioactive, magnetic, quantum dot labels or tags, or the like. In general, any reporter molecule that is detectable can be used. For example, the nucleic acid base is modified to include biotin, which binds to streptavidin that has been previously

covalently linked to a fluorophore. Direct labels are commercially available from several manufacturers, including Boehringer-Mannheim and Amersham-Pharmacia Biotech. Boehringer-Mannheim also sells biotinylated nucleotides, and Amersham-Pharmacia Biotech also sells streptavidin labeled with a variety of fluorophores.

The reporter molecule can be isotopic or nonisotopic, usually non-isotopic, and can be a catalyst, such as an enzyme, a polynucleotide coding for a catalyst, promoter, dye, fluorescent molecule, chemiluminescer, coenzyme, enzyme substrate, radioactive group, a small organic molecule, amplifiable polynucleotide sequence, a particle such as latex or carbon particle, metal sol, crystallite, liposome, cell, etc., which may or may not be further labeled with a dye, catalyst or other detectable group, and the like. The reporter molecule can be a fluorescent group such as fluorescein, a chemiluminescent group such as luminol, a terbium chelator such as N-(hydroxyethyl) ethylenediaminetriacetic acid that is capable of detection by delayed fluorescence, and the like.

The label can generate a detectable signal either alone or together with other members of the signal producing system. As mentioned above, a reporter molecule can be bound directly to a nucleotide sequence or can become bound thereto by being bound to an specific binding pair (sbp) member complementary to an sbp member that is bound to a nucleotide sequence. Examples of particular labels or reporter molecules and their detection can be found in U. S. Patent No. 5,508,178, the relevant disclosure of which is incorporated herein by reference. When a reporter molecule is not conjugated to a nucleotide sequence, the reporter molecule may be bound to an sbp member complementary to an sbp member that is bound to or part of a nucleotide sequence.

Signal Producing System - the signal producing system may have one or more components, at least one component being the label. The signal producing system generates a signal that typically relates to the presence or amount of a target in a medium. The signal producing system includes all of the reagents required to produce a measurable signal. Other components of the signal producing system may be included in the developer solution and can include substrates, enhancers, activators, chemiluminescent compounds, cofactors, inhibitors, scavengers, metal ions, specific binding substances required for binding of signal generating substances, and the like.

Other components of the signal producing system may be coenzymes, substances that react with enzymic products, other enzymes and catalysts, and the like. The signal producing system provides a signal detectable by external means, by use of electromagnetic radiation, desirably by visual examination. Signal-producing systems that may be employed in the present invention are those described more fully in U. S. Patent No. 5,508,178, the relevant disclosure of which is incorporated herein by reference.

Member of a specific binding pair ("sbp member") – one of two different molecules, having an area on the surface or in a cavity that specifically binds to and is thereby defined as complementary with a particular spatial and polar organization of the other molecule. The members of the specific binding pair are referred to as cognates or as ligand and receptor (antiligand). These may be members of an immunological pair such as antigen-antibody, or may be operator-repressor, nuclease-nucleotide, biotin-avidin, hormones-hormone receptors, nucleic acid duplexes, IgG-protein A, DNA-DNA, DNA-RNA, and the like.

Deformable – means a material that can be formed into a variety of shapes without causing material failure, such as unintentional ripping, tearing or puncturing. Moreover, a deformable material can be stretched or collapsed with an application of force using a blunt tool, such as a blunt pin or plunger, from a formed shape without causing material failure. A deformable material is optionally puncturable (or penetrable) with an intentional application of force using a sharp tool, such as a needle. A flexible material is a deformable material that has resiliency and is pliable. An elastomer is a flexible, deformable material with memory. An elastomer may be formed into shapes without stress to the elastomer. Moreover, the formed elastomer essentially will return to its original formed shape after an applied blunt force is removed. The deformable material is usual a film, sheet or layer that may be formed in accordance with embodiments of the present invention. The definitions of deformable, flexible and elastomer, or the like, are further described in co-owned, co-pending U. S. Patent Applications Serial Nos. 10/285,756 and 09/103,604, cited and incorporated by reference *infra*.

Modes for Carrying Out the Invention

The embodiments of the present invention provide a novel method of simultaneously conducting multiple chemical reactions, a reaction assembly apparatus and a kit that are low cost because the embodiments can use pre-existing equipment, materials and well-known techniques to provide a self-contained, gas, liquid and/or fluid tight reaction vessel having multiple closed reaction chambers. In particular, an apparatus for and a method of simultaneously conducting assays of multiple biological samples, such as assays of proteins, nucleic acids, specific binding pairs, and the like, may provide for low cost diagnostic, therapeutic and/or analytical applications, for example. Some embodiments of the method and apparatus of the present invention can be subjected to handling and reaction conditions that are not available to the conventional reaction vessels mentioned above. For example, these embodiments can accommodate such reaction conditions and handling, such as intense mechanical agitation, or otherwise inverting the vessel for mixing reactants, under controlled temperature. These handling and reaction conditions promote or accelerate the chemical reactions within the apparatus.

Moreover, some embodiments provide for the results of the multiple chemical reactions to be analyzed while the reaction vessel apparatus is fully assembled. Further, some embodiments provide for the reaction vessel to be disassembled for analysis of reaction products. Various embodiments of the present invention are particularly useful in the art of biological assays of genes, gene components and products, nucleic acids, proteins, antibodies and antigens, ligands and receptors, or the like, for diagnostic, therapeutic and analytical applications, for example. Depending on the embodiment, the method and the apparatus may use conventional microarrays or conventional microtiter plates in a low cost manner for multiplexing chemical reactions in a gas and/or liquid and/or fluid tight reaction assembly.

An embodiment of the present invention is a reaction assembly apparatus that comprises a plate of wells containing test samples, an array of sets of chemical reactants, and a seal between the plate and the array to create a plurality of sealed reaction cells, which is gas, liquid and/or fluid tight. Another embodiment of the present invention is a method of simultaneously conducting multiple chemical reactions that comprises assembling an array of sets of chemical reactants to a plate of test samples, such that the array covers open ends in the test sample wells of the plate

to form a plurality of closed cells. Each closed cell comprises a set of chemical reactants and a respective test sample. The method further comprises sealing the plate to the array to create one or more of a gas tight, a liquid tight, and a fluid tight seal. The test samples are contacted with the chemical reactants in each closed cell of the sealed reaction assembly to facilitate any reaction between the chemical reactants and test samples. Details of the method and apparatus of the present invention are described further below with respect to specific embodiments.

Embodiments of the reaction assembly apparatus of the invention comprise a plate of wells containing test samples, an array of sets of chemical reactants, and a seal between the plate and the array to create a plurality of sealed reaction cells, which is gas, liquid and/or fluid tight. In some embodiments of the reaction assembly apparatus, the plate of wells is a flexible multi-well plate. A well of a flexible multi-well plate comprises a sidewall, deformable end wall, and an open end. The flexible multi-well plate may have a standard well configuration, such as that for a 96, 384, or 1536-well microtiter plate or a 9 x 26 plate having 234 wells on 2.25 mm centers, for example, depending on the embodiment. Figure 1 illustrates a side view of an embodiment of a reaction assembly apparatus 100 comprising an array substrate 102, a flexible multi-well plate 104, and a seal 106 between the array substrate 102 and the plate 104.

Figure 2 illustrates a cross sectional side view of an embodiment 110 of the flexible multi-well plate 104. The flexible multi-well plate embodiment 110 comprises a rigid or semi-rigid support block 112 with a plurality of throughholes 114 spatially arranged as are the wells of a standard microtiter plate (not all the wells are drawn in Fig. 2). The support block 112 has generally planar opposite surfaces 111 and 113. Each throughhole 114 of the plurality extends from the support block surface 111 through the support block 112 to the support block surface 113. Each throughhole 114 has an opening 115 at the support block surface 111 and an opening 117 at the support block surface 113. The shape of the throughholes 114 may be generally cylindrical and the openings 115, 117 may be generally circular, however other shapes are within the scope of the reaction assembly apparatus and are not a limitation herein.

The flexible multi-well plate 110 further comprises a deformable film 116 in contact with the support block surface 113 and covering the openings 117 of the plurality of throughholes 114. The film 116 is sealed, such as joined or fixed, to the surface 113 at least at perimeters or margins of the openings 117 of the block 112, such that the interface between the film 116 and the block 112 is at least fluid tight. The film 116 is deformable without ripping, tearing or puncturing with an application of blunt force. Portions 119 of the film 116 cover the openings 117 and are deformable within a well space or inward with respect to the throughholes 114 in the block 112. As such, the throughholes 114 form wells 114, each having rigid or semirigid sidewalls 118, an open end 115, and a deformable, closed end wall 119.

Figure 3 illustrates another embodiment of the flexible multi-well plate 120. The plate 120 comprises a planar support block 122, a plurality of throughholes 124, and a deformable film 126, as described above for the block 112, throughholes 114 and film 116 of the plate 110. However, rather than being in contact with a corresponding surface of plate 110, the film 126 is in contact with a surface 121 of the support block 122 in this embodiment. The surface 121 is opposite to a surface 123 that corresponds to surface 113 of plate 110. Furthermore, rather than extend over openings 117 in surface 113, as in plate 110, the deformable film 126 extends into openings 125 on the opposite surface 121 to extend within the throughholes 124 to approximately opposites opening 127. The film 126 is formed to fit into and extend between the openings 125, 127 and so extends in the throughholes 124 using a cold forming process in a method of making the flexible multi-well plate 120 of this embodiment. Once made, the flexible multi-well plate 120 of Figure 3 comprises wells 124 that have an open end 125, deformable sidewalls 128, and a deformable, closed end wall 129 that is accessible from the opening 127 in the support block 122.

The deformable film 116, 126 may be an elastomer, such as a latex or other flexible material, or a nonelastomeric plastic, each of which is deformed to one or both of stretch and collapse with an application of blunt force, for example, without ripping, puncturing or tearing. Depending on the embodiment, the film 116, 126 may have memory such that it returns to its original formed shape and position after an applied force is removed, or alternatively, the film 116, 126 may remain deformed after the applied force is removed. The above-described embodiments of the flexible

multi-well plate 104, other embodiments thereof, methods of making such plate, and the deformable films used are described in more detail in a co-owned, co-pending U. S. patent application Serial No. 09/183,604, originally filed Oct. 30, 1998 (on which a CPA was filed and is now allowed, having U. S. Patent Publication No. US 2003/0138968 A1), which is incorporated herein by reference in its entirety.

Figure 4 illustrates a side view of an embodiment of the reaction assembly apparatus 100, 300 that includes the flexible multi-well plate 110. Figure 5 illustrates a side view of an embodiment of the reaction assembly apparatus 100, 300 that includes the flexible multi-well plate 120. Figures 4 and 5 further illustrate a deformation of the film 116, 126 that facilitates mixing a test sample *A* in the flexible multi-well plate 110, 120 with chemical reactants *B* attached or bound to an array 130 of the reaction assembly apparatus 100, 300. In each embodiment, the film 116, 126 is deformed to an extent with an applied blunt force indicated by the bold arrow to displace the test sample *A* in each well toward the chemical reactants *B* attached to the array 130 until the test samples *A* are in contact (i.e., mixes) with the chemical reactants *B*. As mentioned above, the film 116, 126 may have memory to return to its original shape when the applied force is removed, as illustrated in Figure 4, or remain in a deformed state when the applied force is removed, as illustrated in Figure 5, by way of example only.

The flexible multi-well plate 110, 120 may minimize the quantity of sample *A* required in each reaction well 114, 124 while allowing the samples *A* to be introduced into each well 114, 124 of the plate 110, 120. When the wells 114, 124 are in a normal position (i.e., the films 116, 126 are not deformed at the closed end 119, 129), the well volume is maximized. A small quantity of the test chemical sample *A* is placed into the wells 114, 124 either manually or by automated pipettes. Since the available well volume is larger than the intended fluid volume, there is a reduced risk of overfilling and a reduced risk of spilling the sample into another well. When the wells 114, 124 are deformed (i.e., the film 116, 126 is displaced), the closed end walls 119, 129 of the wells 114, 124 are moved inward (i.e., into the well space) to move the menisci of the test samples *A* closer to the array-bound chemical reactants *B* adjacent to the open ends 115, 125 of the wells 114, 124.

In some embodiments of the reaction assembly apparatus, the array of sets of chemical reactants comprises a multi array device. Figure 6 illustrates a side view of an embodiment of a reaction assembly apparatus 200 comprising a multi array device 202, a plate of wells 204 and a seal 206 between the device 202 and the plate 204, that provides one or more of a gas, liquid and fluid tight seal. The multi array device 202 has an array pattern or spatial arrangement that is complementary to the arrangement of wells in the plate of wells 204 containing test samples. Therefore, the array may have an array pattern corresponding to that of a 96, 384, or 1536-well microtiter plate, a 9 x 26 plate having 234 wells on 2.25 mm centers, for example, or other multi well configuration depending on the embodiment.

Figure 7 illustrates a perspective view of an embodiment 210 of the multi array device 202. The multi array device embodiment 210 comprises a foundation support 212 having a plurality of array sites 214 and a plurality of individual pieces of a flexible array substrate 216 attached to the foundation support 212, with an individual piece of flexible array substrate 216 occupying each array site 214. An array site 214 is a site on the foundation support 212 where an individual piece of flexible array substrate 216 is attached. The foundation support 212 is typically a rigid structure adapted to be used in performing multiple array hybridization experiments in parallel. However, the foundation support may be a flexible structure, depending on the embodiment. Figure 7 illustrates only a portion of the multi array device 210, such that the illustrated foundation support 212 is representative only and in practice, the foundation support 212 typically supports more than the number of array sites 214 illustrated therein.

The foundation support 212 comprises and supports a plurality of prongs 211 arranged in an x-y grid layout in the illustrated embodiment. The prongs 211 are regularly spaced and are positioned to correspond to the wells in a plate of wells, such as that described above. Thus the process for use contemplates mating the foundation support 212 with the plate of wells such that the wells receive individual pieces of a flexible array substrate 216 attached to the prongs 211.

Each prong 211 has a proximal end attached to the support 212 and a distal end distally located from the support 212. The distal end provides at least one of the plurality of array sites 214. Each of the plurality of prongs 211 has an individual

piece of flexible array substrate 216 attached thereto at or adjacent the distal end at the array site 214. The individual piece of flexible array substrate 216 may optionally be omitted on one or more of the prongs 211, if desired.

In Figure 7, the prongs 211 are cylindrical-shaped, however any suitable conformation may be used, that is compatible with a shape of the wells in a corresponding well plate. Moreover, the individual pieces of flexible array substrate 216 in Figure 7 is disc-shaped, although any conformation may typically be used, such as rectangular, square, polygonal, circular, or oval.

The individual pieces of the flexible array substrate 216 may be any flexible array substrate or support that may be produced in the desired conformation. A flexible array substrate is a substrate or support as defined and described in co-pending, co-owned U. S. patent application Serial No. 10/285,756, filed October 31, 2002, and includes the other references described therein, such as co-owned applications U.S. Serial No. 10/032,608 to Lefkowitz et al., U.S. Serial No. 01/037,757 to Schembri, and U.S. Serial No. 10/167,662 to Lefkowitz et al. (all filed on Oct. 18, 2001). All such references are incorporated by reference herein. Individual pieces of the flexible array substrate 216 are typically obtained by cutting larger pieces of a flexible array substrate as may be obtained as described in the previously mentioned sources. In some embodiments, a single sheet of flexible array substrate may be affixed to the foundation support 212. A die having cutting edges corresponding to the positions of the prongs 211 is pressed against the single sheet of flexible array substrate on the foundation support 212 to form the multiple individual pieces of the flexible array substrate 216 affixed to the prongs 211 of the foundation support 212. The die and the 'punched-out' remainder of the sheet of flexible array substrate are removed.

The multi array device 210 further comprises an addressable collection of probes 218 present on each individual piece of the flexible array substrate 216. The probes 218 represent the chemical reactants in the array of sets of chemical reactants, or also referred to herein as chemical sample *B*, second chemical samples or second biological materials. Ultimately, the probes 218 are mixed with the test samples provided in the plate of wells when the reaction assembly apparatus 200 is mated, as described below. In some embodiments, the probes 218 are fabricated on the

individual pieces of flexible array substrate 216 before the individual pieces of flexible array substrate 216 are affixed to the foundation support 212. In other embodiments, the probes 218 are fabricated on the individual pieces of flexible array substrate 216 after the individual pieces of flexible array substrate 216 are affixed to the foundation support 212. Yet another embodiment includes fabricating multiple addressable collections of probes 218 on a single flexible array substrate (e.g., a sheet or web) prior to separation of the single flexible array substrate into the multiple individual pieces of flexible array substrate 216. Moreover in an embodiment, at least one addressable collection of probes 218 is different from at least one other addressable collection of probes 218 present on a different individual piece of flexible array substrate 216, such that different collections of probes 218 may be present on the multi-array device 210 and may be screened in parallel or essentially simultaneously. Depending on the embodiment, the addressable collection of probes 218 are fabricated or synthesized *in situ* on the flexible array substrate or alternatively, the probes 218 are presynthesized and then attached to the flexible array substrate, using well-known methods of synthesis and attachment.

The multi array device 210 optionally further comprises one or more stopping prongs. A stopping prong serves to provide a 'stop' when the multi-array device 210 is mated with a corresponding multi-well plate, such that each of the individual pieces of flexible array substrate 216 on a prong 211 is prevented from contacting a bottom or closed end of a corresponding well in the multi-well plate. Various embodiments of the stopping prong are as described in the above-cited, co-pending application Serial No. 10/285,756, and are incorporated herein by reference. An embodiment of the stopping prong 213 has a shape that includes a shoulder feature 215 that is a constituent of the stopping prong 213. The shoulder feature 215 extends at an appropriate distance radially from the stopping prong 213, wherein the distance is from the distal end to provide a stop to prevent the flexible array substrate 216 from contacting the bottom of a corresponding well in the multi-well plate when mated. In other embodiments, most of the prongs 211, 213 may include such a shoulder feature 215. In these embodiments, the shoulder feature 215 has a conical portion that serves as an aid in centering the prongs 211, 213 in the corresponding wells of the multi-well plate and also serves to retard evaporation from the wells after mating, by closing the open end of the corresponding wells. In some of these embodiments, some of the

shoulder features 215 may be a flexible material that conforms to edge of the open end of the well and/or may comprise a flexible gasket or o-ring to facilitate the retarding of evaporation. Where the shoulder feature 215 is flexible or comprises a flexible gasket that surrounds the edge of the well opening, the shoulder feature 215 facilitates the seal 206 illustrated in Figure 6, for example. In these embodiments, any number of the shoulder features 215 may also provide a stop.

In another embodiment of the reaction assembly apparatus, a reaction assembly apparatus 300 comprises both a flexible multi-well plate 104 and a multi array device 202, each as described above for the plate 110, 120 and the multi array device 210 of the respective reaction assembly apparatus embodiments 100, 200. The embodiments of the reaction assembly apparatus 100, 200, 300 are assembled for conducting multiple chemical reactions simultaneously according to a method 400 of simultaneously conducting multiple chemical reactions.

A flow chart of an embodiment of the method 400 of simultaneously conducting is illustrated in Figure 8. The method 400 comprises providing 410 a plate having a plurality of wells that are spatially arranged in an array pattern, where each well is separated by a well spacing. Wells of the plurality receive a volume of a test sample. The test sample in each well may be the same, or may be different in at least one well of the plate. In some embodiments, the test sample has different characteristics to be evaluated in different wells. A gas volume occupies any space in each well that is not occupied by the test sample. In some embodiments, the provided plate is a flexible multi-well plate 104, 110, 120, 204, as described above.

In some embodiments, providing 410 a plate of wells having a test sample comprises introducing an aliquot of each of the plurality of test samples into individual wells of the multi-well plate, and further introducing a second fluid to each well. The test samples are fluid and the aliquot thereof partially fills a volume of the wells. The second fluid has a mass density different from a mass density of the test samples. The second fluid further has a low static miscibility with the test samples and does not adhere to or interact with the array substrate or its bound chemical moieties. In some of these embodiments, while the test sample fluid is the target material to be evaluated and is typically an aqueous solution, the second fluid may be gas, such as air, nitrogen gas, or the like. One or more defoaming agents such as Dow

Corning® Q7-2587, 30% Simethicone Emulsion USP from the Dow Corning Corporation of Auburn, Michigan may be added where the second fluid is air in order to prevent persistent air bubbles from forming in the aqueous solution where mechanical agitation is performed during contacting 450, described further below.

The method 400 further comprises providing 420 an array of sets of a chemical reactant bound to a surface of an array substrate in a spatial array pattern of features. The array of sets of chemical reactants has an array or feature spacing that is similar to the well spacing of the plate. In some embodiments, the features of the array are organized into addressable sets or addressable subarrays of the sets of chemical reactants. Each subarray comprises an addressable matrix of subfeature locations, for example, of the chemical reactants. The matrix may be rectangular, circular or other overall shape, as along as the subfeatures 115 are addressable. The subarrays are arranged relative to each other to have the array spacing. The subfeatures in each subarray have a subfeature spacing that is a fraction of (or much less than) the array spacing. The sets of chemical reactants at each location on the array or in each subarray can be the same, or can be different in at least one location on the array or in at least one subarray or at least one subfeature within a subarray. In some embodiments, the sets of chemical reactants provide different characteristics to evaluate the test sample at different array or different subarray locations. The array and the array pattern are sized, shaped and arranged to interface with the plate and well array pattern. Depending on the embodiment, the array substrate may be optically transparent. In some embodiments, the array is an embodiment of the multi array device 202, 210 described above.

The method 400 still further comprises assembling 440 together the array and the plate to form the reaction assembly apparatus. Any one of the embodiments of the reaction assembly apparatus 100, 200, 300 may be assembled 440 according to the method 400. The array is placed on the plate, such that an array surface is adjacent to a plate surface and covers the open ends of the wells. The array surface comprises the bound chemical reactants and the plate surface comprises the open ends of the wells. The sets of chemical reactants (i.e., sets of probes) are aligned with the open ends of the wells. The array and the plate are then sealed to provide the reaction assembly apparatus having individual closed chambers at each well location that comprise a set

of chemical reactants and a test sample. The closed chambers are gas and/or liquid and/or fluid tight. The seal can be accomplished in several ways according to the invention, depending on the embodiment. The seal may be any one of the seals 106, 206, 500 illustrated in Figures 1, 6 and 10, depending on the embodiment.

In some embodiments, the seal comprises using an array substrate that is flexible and pliable, and further, applying pressure to the assembly with one or more of mechanical clamps, external fluid pressure and vacuum, for example, to contact the flexible array substrate with the plate to form the reaction assembly apparatus. In an embodiment that uses the multi array device 202, 210, the foundation support 212 may be made of a flexible or composite flexible substrate material, such as that defined and described the above-cited, co-pending application Serial No. 10/285,756. In another embodiment that uses the multi array device 202, 210, the foundation support 212 may be rigid or semi rigid. In any of these embodiments, the seal may further include an adhesive, as described further below.

In another embodiment, the array substrate is a flexible material or film and comprises an adhesive surface. The adhesive surface may be an adhesive coating that is applied uniformly, such that the coating underlies the features of array and does not interfere with array processing and analysis. Alternatively, the adhesive coating may be applied by silk screening and/or masking, such that the area in contact with the sets of chemical reactant features does not have adhesive. As another alternative, the adhesive surface may be an adhesive coating that is applied uniformly, such that the coating overlies the features of the array and permits sufficient transmission of the test sample through the adhesive coating to the chemical reactant features of the array. Such adhesive does not interfere with array processing, chemical reactions and analysis. The adhesive on the surface surrounds the feature locations and provides the seal around each open end of the wells by applying heat, pressure and/or radiation, as mentioned above.

The adhesive characteristics are compatible with the substrate material, especially under the conditions of the assay. Ultraviolet light curable adhesives, which have increased adhesion or bond strength with the application of ultraviolet (UV) light, are one type of adhesive that can be used with the present invention. Such UV curable adhesives include, but are not limited to, Dymax UV cure adhesives from

Dymax Corporation, Torrington, CT. Releasable adhesives, i.e., adhesives that have reduced adhesion with the application of heat, cold or radiation, are useful in an embodiment of the method 400, where the reaction assembly apparatus is later disassembled, as described below. UV releasable adhesives have reduced adhesion when exposed to UV light. Adwill D-Series UV curable dicing tape from LINTEC Corp. of Japan; Furukawa UV-Tape from The Furukawa Electric Co, Ltd. of Japan; and Wacker UV-Tape for Wafer dicing available from Dou Yee Enterprises of Singapore are examples of flexible tapes with UV releasable adhesives that might work with the some embodiments of the invention. UV releasable adhesives also sometimes are commonly called UV curable adhesives, leading to possible confusion between adhesives whose adhesion decreases on exposure to UV light and adhesives whose adhesion increases on exposure to UV light. In this document, a 'UV curable' adhesive means one whose adhesion increases on exposure to UV light, and a 'UV releasable' adhesive means one whose adhesion decreases on exposure to UV light, unless otherwise stated.

Alternatively, the flexible array film with an adhesive surface embodiment of the array substrate may use the same adhesive that is used for the ABI Prism® Optical Adhesive Cover from PE Corporation. See for example, web site http://www.hmc.psu.edu/core/Genetics%20Core/RealTime_PCR/UsingOpticalAdhesiveCovers.pdf. The ABI Prism® Optical Adhesive Cover is essentially an optically transparent flexible film tape that has an adhesive surface protected with a protective backing that is peeled away for attachment to a conventional microtiter plate or conventional reaction assembly. For some embodiments of the invention, the ABI Prism® Optical Adhesive Cover is adapted for use as the array substrate, wherein the protected surface is modified, such that the protected surface is the array surface and the areas corresponding to the array of chemical reactant features are rendered non-adhesive. The chemical reactants may be bound to the non-adhesive areas. In some of these embodiments, this modified optical adhesive tape substrate is assembled on the plate to form a seal in accordance with the manufacturers instructions, for example, as provided on the web site. In other embodiments, the same adhesive used for the ABI Prism® Optical Adhesive Cover is uniformly placed on the surface of a flexible tape, and the features of array overlie the adhesive. As a further embodiment, the adhesive used for the ABI Prism® Optical Adhesive Cover is placed on a rigid

substrate, either only in sealing areas, which are removed from the areas of features, or in a uniform film underlying both the sealing areas and the areas of features.

In still another embodiment, the seal may comprise using any conventional substrate material for the array substrate and further using a flexible and/or pliable gasket between the array substrate and the plate. Reference numerals 106, 206, 500 refer both to a gasket and generally to a seal in Figures 1, 6 and 10 herein unless stated otherwise. A seal herein may or may not include a gasket, depending on the embodiment. The conventional array substrate is basically nonreactive in the chemical reactions between the chemical samples and include such materials as silica glass, soda lime glass, or a polymeric material, all such materials being well known in the art and further defined in the Definitions section. In some embodiments, the array substrate is optically transparent. A gasket useful herein has a plurality of throughholes in a throughhole pattern that is similar to and aligns with the feature array pattern and the well array pattern. In yet another embodiment, the pliable gasket material may be integral to the surface of the plate that is adjacent to the well open ends. For example, the flexible multi-well plate 120 has a flexible film 126 on the plate surface 121 that interfaces with an array substrate when assembled 440. Such film 126 may function as the integral gasket in some embodiments. In such gasket embodiments, a seal is made by applying pressure to compress the gasket material against the array substrate and/or the plate using one or more of mechanical clamps, external fluid pressure and vacuum, for example. Depending on the embodiment, the gasket may comprise an adhesive surface such that the seal is made by further applying one or more of radiation, heat and cold.

When a gasket is used to facilitate the seal, the gasket throughholes are aligned with respective openings of the wells. The wells are then capped, covered or enclosed by placing the array over the gasket, such that the array surface adjacent to the chemical reactants is facing the gasket and the features or subarrays are aligned with respective openings of the wells and the gasket throughholes. Depending on the embodiment, the reaction assembly apparatus is sealed to provide a gas and/or liquid and/or fluid tight seal by compressing the gasket between the plate of wells and the array with mechanical clamps, with external fluid pressure, or with vacuum, or is sealed using an adhesive with the gasket, for example.

For the method 400, other examples of adhesive seals include one or more of applying an adhesive around the perimeter of the openings of the wells, around the perimeter of the features, around the perimeter of each through hole in the gasket (and each channel, where applicable), around the perimeter of the plate and array, and the perimeter of both major sides of the gasket and still be within the scope of the various embodiments of the present invention. For adhesives requiring a cure step to activate the adhesion, after the adhesive is cured any clamps or other application of pressure can be removed.

In some embodiments that use a gasket to seal the reaction assembly, the gasket provides a seal around each well opening to form closed cells, so that the chemical samples in one well will not mix with the chemical samples in an adjacent well, and further, will not react with an unaligned feature or subarray, when mechanically agitated, as described further below. However, in another embodiment, a seal is provided by a gasket 500 that has at least one through hole 504 that is interconnected with at least one other through hole 504 via channels 503, 505 in the gasket 500, as illustrated in a plan view of a portion of a major surface of the gasket 500 in Figure 9. During mechanical agitation, the test chemical samples contained in respective wells that are aligned with the interconnected throughholes 504 will mix together and react with features or subarrays that are aligned with the interconnected throughholes 504, as is further described below.

In addition to the flexible or pliable gasket material, in still another embodiment, the seal may further comprise an adhesive on at least one of the major surfaces of the gasket to facilitate assembly of the reaction assembly apparatus. The adhesive surfaces can be applied as a coating or the like, using an adhesive selected from those described above for the method 400. In other embodiments, the gasket itself can be made of a flexible adhesive film that may or may not have reinforcing material, such as a fiber mesh, similar to the adhesive films or tapes manufactured for the electronics industry by Ablestik Laboratories, Rancho Dominguez, CA.

The method 400 still further comprises simultaneously contacting 450 the test samples to the sets of chemical reactants for a period of time in the plurality of closed cells or chambers formed by assembling 440 the apparatus. Contacting 450 facilitates mixing and any reactions that may occur between the sets of chemical reactants and

the test sample. The reaction parameters used in contacting 450 vary and depend on the chemical samples used and reaction conditions needed for accomplishing the chemical reactions. Further, the parameters for contacting 450 may depend on the embodiment of the reaction assembly apparatus. In some embodiments, contacting 450 comprises inverting the reaction assembly apparatus, such that the test sample in each well may contact the chemical reactants on a respective aligned array feature in the closed cell. Gravity will tend to pull the test sample volume of each well into contact with the respective aligned feature of the chemical reactant. In other embodiments, contacting 450 comprises taking the reaction assembly apparatus to a desired reaction temperature, which is maintained for the period of time. The reaction assembly apparatus may or may not be inverted when the temperature is changed.

For the reaction assembly 100, 300 embodiments, the deformable film 116, 119, 126, 129 of a well is displaced with an applied force, such as using a blunt plunger or pin tool, to mechanically move or displace the test sample in the well toward the chemical reactants bound to the array, as necessary. The pin tool may be a pin array bed with a spatial arrangement of blunt pins that correspond to the spatial arrangement of wells in the flexible multi-well plate 110, 120. Moreover, the pin array tool may be automated to either simultaneously or sequentially activate the pins to displace the film portions 119, 129 in each well, depending on the experiment. Likewise, the pin array tool may be automated to activate the pins to displace the film one or more of in a repetitive motion and in an iterative motion. The pin array tool also may have more than one pin per respective well, such that the pins per well move iteratively and repetitively with respect to one another to displace and mix the contents of a respective well with the chemical reactants. When the deformable film is elastic (i.e., having memory), the film can be moved back and forth with the action of the pins to displace the fluid in the sealed well chamber to facilitate mixing.

In embodiments that use the deformable or flexible film 116, 126, contacting 450 may further comprise taking the reaction assembly apparatus 110, 300 to a desired reaction temperature, which is maintained for the period of time. The flexible film 116, 126 is able to distort under the induced pressure from the liquid test sample, thus lowering the pressure on the reaction assembly seal. Moreover, a volume change may occur in the closed chamber due to a chemical reaction occurring during mixing,

either in combination with heating or separate from heating. The flexible film is able to accommodate the volume change, whether the volume change comprises a volume increase or decrease.

Depending on the embodiment, the reaction assembly apparatus 100, 300 may be placed on the pin array tool using one or more alignment pins or markings to ensure properly alignment of the wells over the pins. After the apparatus is aligned on the pin array tool, the assembly is temporarily secured to the tool. To contact 450 the chemical reactants on the array with the test samples in the wells, the pins would be activated accordingly. During activation, the reaction assembly would remain secured to the pin array tool, such that the movement is essentially restricted to within the wells.

In still another embodiment, contacting 450 may further comprise mechanical agitating the reaction assembly or at least the fluids in the closed cells. The reaction assembly apparatus is mechanically agitated to facilitate mixing and/or chemical reactions within each closed cell. How the reaction assembly apparatus is mechanical agitated may depend on the embodiment thereof. For example, the movement of pins on a pin tool or plate to displace the flexible or deformable film 119, 129 in each well of the reaction assembly apparatus 100, 300 is a form of mechanical agitation in some embodiments. The movement of the pins essentially mixes the test sample with the chemical reactants in each closed chamber. In some embodiments, the reaction assembly apparatus may or may not be inverted for the agitation. In yet still another embodiment, the reaction assembly apparatus is inverted, taken to the desired reaction temperature for the period of time and mechanically agitated to provide optimum mixing and reaction conditions. Mechanical agitation can decrease the reaction time without loss of sample in the reaction assembly apparatus of the present invention.

According to some embodiments, contacting 450 comprises exposing the biological materials in the reaction assembly apparatus to hybridization or binding conditions known in the art. The reaction assembly apparatus embodiments of the present invention can be exposed to intense mechanical agitation without loss of sample to accelerate any reactions between biological materials under a controlled temperature in accordance with the method 400.

Mechanically agitating the reaction assembly apparatus facilitates differences in the mass densities of the test sample and the second fluid to cause mixing of the test samples with the chemical reactants in each closed cell. Mixing of the test samples and chemical reactants produce reaction products used to evaluate the test samples. In some embodiments, agitating is performed under controlled temperature conditions. Mechanical agitation will increase the reaction rate between the test samples and the chemical reactants. Examples of conventional equipment that can provide automatic mechanical agitation for an assay include, but are not limited to, the Lab-Line Model 4625 Titer Plate shaker from subsidiary Barnstead-Thermolyne of Apogent Technologies, Portsmouth, NH; the Model 51402-00 Microwell Plate Orbital Shaker with Timer manufactured by the Cole-Parmer Instrument Company of Vernon Hills, Illinois; and Model 400 hybridization incubator with rotisserie built inside for tumbling originally manufactured by Robbins Scientific of Sunnyvale, California, now Matrix Technologies. Similar hybridization incubators have Agilent part number G2505-80081 (for 220-240 volts) and Agilent part number G2505-80082 (for 110 volts), from Agilent Technologies, Inc., Palo Alto, California.

However, because each chamber in the reaction assembly apparatus is sealed, some embodiments can employ much more violent or intense agitation than the agitation provided by the above standard equipment. The sealed reaction assembly apparatus can be exposed to agitation, which is more on the order of the agitation provided by a one-gallon paint shaker, such as the Speed-I-Mix Model CX Explosion-proof single head power paint shaker made by Fleming Gray Limited of St. George, Ontario, Canada, and available from Certified Technology Inc. of Niagara Falls, New York (see the web site at http://www.certifiedtechnology.com/can/fleming.html). In practice, the method 400 of the present invention can use custom-built shaking equipment providing more violent or intense agitation than the standard laboratory equipment, but on a smaller scale than the one-gallon paint shaker.

In an embodiment where the seal comprises using the gasket 500, the gasket 500 is used to interconnect closed cells to provide fluid communication between the interconnected closed cells during agitation. In some embodiments, the cells to be interconnected are adjacent. Figure 10 illustrates a side view of the reaction assembly apparatus 100, 200, 300 having the gasket 500 illustrated in Figure 9 as the seal 500.

The reaction assembly apparatus can be mechanically agitated with incrementally increasing acceleration, to effect sequential mixing of the interconnected closed cells formed by the channels 503, 505 in the gasket 500. Consider, for example, a first cell C_1 and a second adjacent cell C_2 filled with respective first aqueous samples *L* and *M*, and interconnected by a first channel 503 with hydrophobic walls and with a nominal hydraulic diameter designated by a value *x*, as illustrated in Figures 9 and 10. In the presence of gentle agitation with a peak acceleration level D_1, the first samples *L* and M are agitated within the first and second cells C_1, C_2, respectively, but the first sample *L* does not enter the second cell C_2, nor does the first sample *M* enter the first cell C_1, because the hydrophobic nature of the first channel 503 prevents each of the first samples *L* and *M* from transiting the first channel 503. However, as the acceleration due to agitation is next increased to a peak acceleration level D_2 that is greater than D_1, the inertial forces imparted to the first samples *L* and *M* cause them to breach the hydrophobic barrier and transit repeatedly across the first channel 503, causing mixing of the first samples *L* and *M* in the first cell C_1 and second cell C_2, thus facilitating any chemical reaction which may occur between the first samples *L* and *M*.

Consider further a third cell C_3 adjacent to the second cell C_2, the third cell C_3 containing a first sample *N*, and the third cell C_3 being interconnected to the second cell C_2 by a second channel 505 with hydrophobic walls and with a nominal hydraulic diameter designated by *y*, where *y* is less than *x*. The hydrophobic nature of the second channel 505 prevents the first samples *M* and *N* from intermixing under gentle agitation with a peak acceleration level D_1. Because the diameter *y* is less than the diameter *x*, the hydrophobic nature of the second channel 505 also prevents the contents of the third cell C_3 from intermixing with the intermixed first samples *L* and *M* in the presence of the agitation with a peak acceleration level D_2, which was sufficient to mix the first samples *L* and *M*, but insufficient to cause transit of samples across the second channel 505. However, as the acceleration due to agitation is increased to a peak acceleration level D_3, which is greater than acceleration D_2, mixing occurs between the first sample *N* in the third cell C_3 and the intermixed first samples *L* and *M*, thus facilitating any chemical reaction which may occur between the first sample *N* and the intermixed first samples *L* and *M*.

The above described agitation using interconnecting channels 503, 505 of different hydraulic diameters x and y can be extended to greater than three interconnected cells. Agitating provides a method of sequentially mixing or intermixing samples in the presence of increasing levels of acceleration due to agitation. It will be appreciated by those skilled in the art that the channels 503, 505, which provide a barrier to liquid transit, can also be created using channels with hydrophilic walls rather than hydrophobic walls, as long as the geometry of the hydrophilic walls is such as to provide a potential barrier to liquid intrusion. For a discussion of the physics of liquid and gas behavior in hydrophilic and hydrophobic channels of variable hydraulic diameter, see, for example, European patent application EP 1 014 140 A2, entitled "Capillary Fluid Switch," which is hereby incorporated by reference in its entirety herein. Also, see U. S. Patent No. 5,472,603 entitled "Analytical Rotor with Dye Mixing Chamber", which is incorporated by reference herein in its entirety.

In some embodiments, the chemical reactions are quenched after the reaction time period. Depending on the embodiment, quenching can comprises removing the reaction temperature, where a temperature change was applied; or stopping the mechanical agitation where agitation was applied; or both removing and stopping, where both temperature and agitation were applied, for example.

In some embodiments, the array, having bound reactions products as a result of hybridization during an assay, is washed to remove unreacted or unbound test sample from the array surface in preparation for subsequent analysis, described below. After hybridization is complete, the array surface is washed to remove the unbound test sample and to remove any residual non-specifically bound signal. Moreover, the array surface is kept wet until the washing process is complete. If any unreacted or unbound labeled test sample dries on the array surface, it is often extremely difficult to remove by subsequent washing. The residual, dried labeled test sample will produce background noise or signal during subsequent analysis, which compromises one or both of the assay integrity and the assay low-end sensitivity. The materials and methods used for rinsing or washing the reacted array are well known in the art.

The flexible multi-well plate 104, 204 of the reaction assembly apparatus 100, 300 can assist in a wash portion of the assay. In embodiments that use a pin tool or

plate to mechanically agitate the closed cells of the reaction assembly apparatus 100, 300, the pin plate is retracted at the conclusion of the hybridization. One or more needles having a sharp tip are used to deliberately puncture or penetrate the thin deformable film 116, 119, 126, 129 at the well ends 117, 127 and to introduce a first wash fluid into the punctured cells or wells 114, 124 to dilute the test sample and hybridization buffer included therein. The buffer and sample may be removed by displacement or vacuum through a second needle or an exit port formed in the deformable film 116, 126. Due to the injection of the first wash fluid, the deformable film 116, 126 may expand causing the well to enlarge if the injection speed is faster than the removal of any fluid. This expansion can alternatively cause fluid to exit through the same puncture hole caused by the needle in the deformable film as the hole expands around the needle by fluid pressure on the film. In other embodiments, the deformable film 119, 129 can be deliberately torn or cut away from the well support block 120, 220 with a tool having a sharp edge either instead of puncturing or in addition to puncturing the film. In some embodiments, a large amount of wash fluid is passed through the well to remove residual test sample to ensure that test samples cannot be exposed to different wells during a sample removal portion of an array washing protocol.

Several options exist for continued array washing according to the array washing protocol, since it is typical for several wash buffers to be employed in sequence to complete the assay. First, different wash fluids can be sequentially introduced into the punctured well through the first needle and drained through the second needle or the exit port. Second, the initial washing may sufficiently clean the array to allow it to be momentarily removed from the flexible multi-well plate and moved to a second well plate or large volume bath for continued washing without undue concern for the sample (e.g., unreacted test sample) drying on the array surface.

This washing protocol works in either flexible multi-well plate 110, 120 with any of the disclosed arrays 102, 130, 202, 210 and is especially helpful in an automated, high throughput hybridization laboratory.

In another embodiment, the reaction assembly apparatus is disassembled to rinse or wash the surface of the reacted array. According to some embodiments, the reaction assembly apparatus of the present invention may be disassembled without

damage to the parts. Where pressure was used to form the seal, the apparatus disassembly is a matter of removing the pressure source. Where a UV releasable adhesive was used to form the seal, the apparatus disassembly comprises applying UV light to the adhesive to release the array substrate from the plate. Depending on the nature of the first and second samples, it may be necessary to use a shadow mask to shield the features from the UV light source during the application of UV light. Such a shadow mask can take the form of, for example, thin film chrome shadowing features on a transparent fused quartz plate, which is well known to those skilled in photolithographic techniques for the semiconductor circuit fabrication art.

In some embodiments, the method 400 further comprises analyzing 460 the array. In some of these embodiments, the array may be analyzed 460 after the apparatus is disassembled and the array surface is rinsed. In other of these embodiments, the array can be analyzed 460 while the reaction assembly apparatus is still assembled, as long as either the array substrate or the plate is transparent to the radiation used for analysis, or if a radioactive source is used and the array substrate is thin. Analyzing 460 comprises interrogating the array and processing data collected as a result of the interrogation. The array is analyzed 460 to determine information about any reaction products on the surface of the reacted array.

Typically, analysis is performed with automated equipment that can address the feature locations (and subfeature locations in a subarray) individually, sequentially and/or simultaneously to evaluate any reaction products at each location. The automated equipment may include a radiation source and detector interfaced with a computer or microprocessor that provides for scanning the array. The radiation source is used to interrogate the array. The radiation source may emit electromagnetic, radioactive particle, or ultrasonic radiation, for example. Typically, either the first chemical sample or the second chemical sample will be labeled with a label or tag that emits a signal when scanned or interrogated with the particular radiation source. Conventional labeling materials and techniques that are well-known in the art can be used to label the chemical samples in accordance with the invention (also see Definitions section above). Further, conventional scanning equipment that is well-known in the art can be used for the invention, for example the GENEARRAY scanner manufactured by Agilent Technologies, Inc. of Palo Alto, CA (also see e.g.,

web site http://www.bioelectrospec.com or http://barolo.ipc.uni-tuebingen.de/infomat/tirf/rianaindex_en.html for information on 'total internal reflection fluorescence' (TIRF), which may be used in the analysis aspect of the present invention). Alternatively, if a radioactive label such as phosphorous is used to label the first chemical sample, for example, a phosphor imager such as one of those manufactured by Packard Instruments or Fujifilm or Molecular Dynamics or others may be used, and an external radiation source is not needed. However, it is not the intent of the inventors to be limited to any particular labeling system and scanning equipment. All labeling systems and scanners known in the art are within the scope of the embodiments of the invention.

The scanning system will interrogate and process signal data obtained from scanning the tags present on the array after contacting 450. The radiation will elicit responses or signals from the labeled reaction products, which are detected by the detector and analyzed by the computer or microprocessor. The intensity of each signal and its locations on the array will provide valuable information about the chemical samples and reaction products.

The reaction products produced in the reaction assembly apparatus by the method 400 can be analyzed 460 using techniques and equipment described above. For the purposes of some embodiments, a first chemical sample or test sample is a first biological material, such as an oligonucleotide or polynucleotide, a protein, enzyme, polypeptide, antibody, antigen, ligand, receptor, polysaccharide, carbohydrate, or the like, and a second chemical sample or chemical reactant is a complementary second biological material to the first biological material. Either the first material or the second material is used to evaluate the other. For the purpose of simplifying this discussion, the first biological material will be evaluated by the second biological material in a binding assay, such that complementary oligonucleotides, antibody/antigens, ligand/receptors, and the like, are allowed to interact.

In another embodiment, the first biological material is a target or test material, such as an antigen or an oligonucleotide or polynucleotide that is to be evaluated by the second biological material, which is a probe, such as an antibody or an oligonucleotide probe having a known makeup or sequence. The antibody probe or oligonucleotide probe is complementary to the target antigen or target

oligonucleotide, respectively. A plurality of different probes may be bound to the array substrate in order to extensively evaluate the composition of the target. For oligonucleotide assays, in some embodiments there is more than one different target oligonucleotide or different portions of a target polynucleotide to be evaluated and a plurality of different oligonucleotide probes arranged on the array that are used to evaluate the different target oligonucleotides or portions. The different probes of the plurality are complementary to different target oligonucleotides or portions.

Another embodiment of the present invention is a kit that comprises one or more of an array 102, 202, 210 of biological features, a plate 104, 204, 110, 120 for introducing a sample under test, a gasket 106, 206, 500, mechanical clamps, an adhesive such as that mentioned above, and a sample biological material for a control experiment. Optionally, the kit further comprises instructions in the form of the method 400 of simultaneously conducting multiple reactions of a test sample using the reaction assembly apparatus 100, 200, 300 of the present invention.

For the method 400, a user might assemble 440 a prefabricated array of probes with a microtiter plate of target samples into the reaction assembly apparatus of some embodiments of the present invention for a hybridization assay to determine information about the target samples. The user might label the target samples with one or more fluorescent labels, for example, using conventional methods prior to assembly, then contact 450 the probes with the target samples, such as by hybridizing them, and subsequently, interrogate and process the hybridized array to analyze 460 the reaction products using well-known conventional methods and equipment. The interrogation will produce a result. Information about the target sample(s) can be obtained from the results of the interrogation. Interrogation is usually accomplished by a suitable scanner, as described above, which can read or detect the location and intensity of fluorescence (signals) at each feature of the array. For example, such a scanner may be similar to the GENEARRAY scanner mentioned above. Results from the interrogation can be analyzed results. For example, results conventionally obtained by rejecting a reading for a feature that is below a predetermined threshold are analyzed results in some embodiments. Moreover or alternatively, results conventionally obtained by forming conclusions based on the pattern read from the

array (such as whether or not a particular target sequence may have been present in the sample) are analyzed results depending on the embodiment.

A user might manufacture or purchase the array and/or the plate, that user or another user might assemble the reaction assembly apparatus, and either of these users or still another user might perform the hybridization assay at the same or remote locations and still be within the scope of the present invention. Yet another user might perform the interrogation and analysis at a location remote to the hybridization location. The results of the interrogation (analyzed or not) can be forwarded (such as by communication) back to the first-mentioned user, or to another remote location if desired, and received there for further use by the first or second user or still another user. Moreover, the user(s) may be in a location(s) remote to the location where the method 400 is performed or the array is fabricated. A user may communicate or forward the results, or the information obtained from the results, to a location remote to the user's location and still be within the scope of various embodiments of the present invention. The definitions of 'user', 'remote', 'communicating' and 'forwarding' provided in the above-cited parent U. S. patent application Serial No. 09/938,909 applied equally herein.

Thus, there have been described a novel method for simultaneously conducting multiple chemical reactions, a reaction assembly apparatus that is used to conduct the multiple reactions, and a kit comprising one or more components of the apparatus and method. It should be understood that the above-described embodiments are merely illustrative of some of the many specific embodiments that represent the principles of the present invention. Clearly, those skilled in the art can readily devise numerous other arrangements without departing from the scope of the present invention.